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PCT

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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(54) Title: IMMUNOGENIC PEPTIDES		
<p>(57) Abstract</p> <p>The instant invention relates to immunogenic synthetic peptides comprising amino acid sequences corresponding to antigenic determinants of the capsid of HAV, advantageously, linked directly or through a spacer molecule to carrier molecules suitable for vaccination of mammals.</p>		

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IMMUNOGENIC PEPTIDESBACKGROUND OF THE INVENTIONTechnical Field

The instant invention relates to vaccines  
5 for the prevention of hepatitis A viral (HAV)  
infection and, in particular, to vaccines comprising  
synthetic peptides having an amino acid sequence  
homologous to a portion of the amino acid sequence  
of a neutralization immunogenic site of the HAV  
10 capsid.

BACKGROUND INFORMATION

HAV accounts for 38% of the cases of viral  
hepatitis reported annually in the United States  
(D.P. Francis et al, Am. J. Med. 76:69-74 (1984)).  
15 On the basis of morphologic and biophysical  
characteristics, HAV has been classified among the  
Picornaviridae (I.D. Gust et al, Intervirology  
20:1-7 (1983)). Like poliovirus, the virion is a  
naked icosahedron. The capsid contains at least  
20 three major structural proteins (VP1, VP2 and VP3),  
of which VP1 was previously believed to be the  
dominant surface protein (A.G. Coulepis et al,  
Intervirology 18:107-127 (1982)). Only a single  
antigenic specificity has been associated with HAV,  
25 and significant antigenic variation has not been  
recognized among different HAV strains (S.M. Lemon  
et al, Infect. Immun. 42:418-420 (1983)).  
Denaturation of the virus leads to a loss of  
antigenicity, suggesting that the relevant antigenic  
30 sites are strictly conformationally dependent (J.V.  
Hughes et al, J. Virol. 52:465-473 (1984)). By

covalently cross-linking monoclonal antibodies to intact HAV, one major antigenic site has been located on VP1 (J.V. Hughes et al, J. Virol. 52:465-473 (1984)).

5           Analysis of mutants that are resistant to neutralizing monoclonal antibodies has proven useful for identifying immunogenic sites involved in the neutralization of other picornaviruses (D.C. Diamond et al, Science 229:1090-1093 (1985); P.D. Minor et al, J. Gen. Virol. 65:1159-1165 (1985); B. Sherry et al, J. Virol. 53:137-143 (1985)). Genomic  
10           nucleotide substitutions associated with neutralization resistance, when analyzed in the context of the three-dimensional structure of  
15           poliovirus type 1 (PV1) or human rhinovirus type 14 (HRV14), have identified multiple discrete sites on the virion surface which appear to function both as immunogens and as attachment sites for neutralizing monoclonal antibodies (J.M. Hogle et al, Science  
20           229:1358-1365 (1985); M.G. Rossman et al, Nature (London) 317:145-153 (1985)). Four such neutralization immunogenic sites have been identified on both PV1 and HRV14; they involve all three surface polypeptides (J.M. Hogle et al,  
25           Science 229:1358-1365 (1985); B. Sherry et al, J. Virol. 57:246-257 (1985)). In general, mutation occurring within one neutralization immunogenic site has conferred resistance to all monoclonal antibodies binding at that site, but not to those  
30           binding at alternate sites.

          At present, no vaccine is available for prevention of infection with HAV. Because HAV closely resembles poliovirus and can be grown in cell cultures, intensive efforts over the past few  
35           years have been directed at the development of

inactivated and/or live attenuated HAV vaccines.  
For a variety of technical reasons (including  
difficulties in achieving high yields of virus in  
cell cultures), a clinically useful vaccine for  
5 prevention of infection with this virus has not yet  
been developed.

#### SUMMARY OF THE INVENTION

It is a general object of the instant  
invention to provide synthetic peptides that are  
10 capable of inducing the production in mammals of  
neutralizing antibodies against HAV.

It is another object of the invention to  
provide vaccines comprising a synthetic peptide(s)  
having an amino acid sequence corresponding to an  
15 antigenic determinant of the HAV capsid that is  
capable of inducing protective immunity in mammals  
against HAV.

It is an additional object of the invention  
to provide a method of detecting the presence of HAV  
20 antibodies in biological test samples.

These, and other objects which will be  
clear to those skilled in the art from the following  
detailed description, have been accomplished by  
providing synthetic peptides useful in producing an  
25 immunogenic response to the viral causative agents  
of HAV.

The invention relates to immunogenic  
preparations and vaccines made therefrom. Vaccines  
of the instant invention comprise a synthetic  
30 peptide(s) having an amino acid sequence  
corresponding to an antigenic determinant of the VP3  
structural protein of the capsid of HAV,  
advantageously, linked to a suitable carrier  
molecule.

In one embodiment, the instant invention comprises a synthetic peptide having an amino acid sequence homologous to a portion of the amino acid sequence of a neutralization immunogenic site of the VP3 structural protein of the capsid of HAV, which peptide is capable of inducing in a mammal neutralizing antibodies against HAV. The synthetic peptide(s) of the instant invention corresponds to the region of VP3 containing the aspartate (asp)-70 residue.

In another embodiment, the instant invention comprises a DNA segment encoding the above-described peptides.

In a further embodiment, the instant invention comprises an immunogenic conjugate capable of inducing in a mammal neutralizing antibodies against HAV. The conjugate comprises a synthetic peptide having an amino acid sequence corresponding to an antigenic determinant of the VP3 structural protein of the capsid of HAV.

In yet another embodiment, the instant invention comprises a method of producing immunity to HAV comprising administering the above-described conjugate to a mammal.

In another embodiment, the instant invention comprises a method of detecting the presence of HAV antibodies in biological test samples.

#### BRIEF DESCRIPTION OF THE DRAWING

FIGURES 1A and 1B. Nucleotide sequence of VP1 of HMI75 of HAV.

FIGURES 2A and 2B. Nucleotide sequence of  
VP3 of Hm175 of HAV.

FIGURE 3. Competition between monoclonal  
antibodies for attachment to HAV fixed to a solid-  
5 phase support.

FIGURE 4. Map of genomic regions from  
various HAV neutralization-escape mutants sequenced  
by the dideoxynucleotide method.

FIGURE 5. Resistance of mutant S30 to  
10 neutralization by 9 different monoclonal antibodies  
to HAV.

#### DETAILED DESCRIPTION OF THE INVENTION

The instant invention relates to synthetic  
peptides, and DNA segments encoding same,  
15 corresponding to immunogenic epitopes of HAV and  
vaccines made therefrom. These novel immunogenic  
agents are prepared by synthesizing, either  
chemically or by recombinant techniques, peptides  
having an amino acid sequence corresponding to, or  
20 homologous with, B-cell epitopes present on the VP3  
structural protein of the capsid of HAV. The  
peptides of the instant invention correspond to the  
region of VP3 containing the asp residue at position  
70. The peptides, advantageously, linked to an  
25 appropriate carrier molecule, evoke the production  
in mammals of high titers of neutralizing antibodies  
against HAV. Vaccines thus formed are useful for  
immunization against HAV infection when administered  
to mammals, for example, intramuscularly,  
30 subcutaneously or intradermally, advantageously,  
intradermally.

SUBSTITUTE SHEET



To topologically map immunogenic sites on HAV which elicit neutralizing antibodies, eight neutralizing monoclonal antibodies were evaluated in competition immunoassays employing radiolabelled monoclonal antibodies and HM-175 virus according to procedures described in J. Virol., Vol. 61, No. 2, Feb. 1987, pp. 491-498 (the entire contents of which reference is hereby incorporated by reference and relied upon). Whereas two antibodies (K3-4C8 and K3-2F2) bound to overlapping epitopes, the epitope bound by a third antibody (B5-B3) was distinctly different as evidenced by a lack of competition between antibodies for binding to the virus. The other five antibodies variably blocked the binding of both K3-4C8--K3-2F2 and B5-B3, suggesting that these epitopes are closely spaced and part of a single neutralization immunogenic site. Several combinations of monoclonal antibodies blocked the binding of polyclonal human convalescent antibody by greater than 96%, indicating that the neutralization epitopes bound by these antibodies are immunodominant in humans.

Virus mutants resistant to monoclonal antibody-mediated neutralization have proven helpful in identifying immunogenic sites on other picornaviruses (D.M.A. Evans et al, Nature 304:459-462 (1983); P.D. Minor et al, J. Gen. Virol. 67:1283-1291 (1986); B. Sherry et al, J. Virol. 53:137-143 (1985)), but the application of this approach to characterization of HAV antigenic sites has been hampered by slow and inefficient virus replication in vitro. Most HAV isolates are noncytopathic, highly cell-associated, and demonstrate a substantial nonneutralizable fraction in vitro (S. M. Lemon, N. Engl. J. Med. 313:1059-

- 1067 (1985); J.R. Ticehurst, Seminars in Liver Dis. 6:46-55 (1986)). To overcome these problems, a radioimmunofocus assay was developed for HAV. The assay was based on the immune autoradiographic
- 5 detection of virus replication foci developing beneath agarose overlays. The method was modified for clonal isolation of virus variants (S.M. Lemon et al, J. Clin. Microbiol. 17:834-839 (1983); S.M. Lemon et al, J. Virol. Methods 11:171-176 (1985)).
- 10 A quantitative neutralization assay, based on the radioimmunofocus assay (S.M. Lemon et al, J. Infect. Dis. 148:1033-1039 (1983)), demonstrated that extraction of virus with lipid solvents substantially reduces the nonneutralizable virus
- 15 fraction (S.M. Lemon et al, J. Gen. Virol. 66:2501-2505 (1985)). Nonetheless, most clonally isolated virus variants recovered from agarose overlays following stringent monoclonal antibody-mediated neutralization reactions demonstrated continuing
- 20 neutralization susceptibility. The selection of spontaneous neutralization-escape mutants of HAV required a process of repeated neutralization and passage in the presence of antibody, followed by clonal isolation from agarose overlays (J.T. Stapleton et al, J. Virol. 61:491-498 (1987)).
- 25 Neutralization resistance of such mutants is associated with reduced binding of antibody to the virion (J.T. Stapleton et al, J. Virol. 61:491-498 (1987)). To identify capsid protein amino acid
- 30 mutations associated with neutralization resistance, and by inference sites involved in antibody attachment on the virion surface, methods for RNA sequencing (D.M.A. Evans et al, Nature 304:459-462 (1983); F. Sanger et al, Proc. Nat. Acad. Sci. USA
- 35 74:5463-5467 (1977)) were modified to permit the

sequencing of as little as 2 to 10 ng of template RNA.

Mutant S30, selected by neutralization and passage in the presence of monoclonal antibody K2-4F2, showed no decrease in titer when incubated with this antibody (J.T. Stapleton et al, J. Virol. 61:491-498 (1987); see Table 1). The entire capsid-encoding region of S30 RNA was sequenced by primer-extension (see Example 2). Its sequence was then compared with that obtained from cDNA clones of parental virus with normal neutralization phenotype, and with the sequence of S32 virus. This variant was selected against monoclonal antibody B5-B3 but had substantially lost neutralization resistance during amplification in the absence of antibody.

The only mutation identified in the capsid-encoding region of the neutralization-resistant S30 virus was a G to C substitution at base 1677, predicting a change in asp-70 of capsid protein VP3 to his. This substitution also was present in two other mutants selected against K2-4F2, S18 and S27, but absent in mutant S20 which had reverted to neutralization susceptibility during final large volume cell culture passage (see Table 1). Because of the method of mutant selection (J.T. Stapleton et al, J. Virol. 61:491-498 (1987)), these viruses are believed to represent sibling clones derived from a single neutralization-resistant parent. Asp-70 was unchanged in S32, and also in S1, S33, S34 and S57. The S1, S33, S34 and S57 mutants were initially selected for resistance to other antibodies but had reverted to neutralization susceptibility during passage in the absence of antibody. In variant S32, a C to T mutation was evident at base position 2512, predicting a change in ser-102 of VP1 to leu. S32

demonstrated significant neutralization susceptibility after amplification in the absence of antibody (see Table 1).

Table 1

## 5 Neutralization Resistance of HAV Variants

	Cognate Antibody	Mutant	Neutralization	Control
			Index <sup>1</sup>	Neutralization Index <sup>2</sup>
10	K2-4F2	S30	-0.09	1.33
		S18	-0.12	1.53
		S20	0.88	1.80
		S27	-0.06	1.53
15	K3-4C8	S1	0.92	1.66
		B5-B3	0.65	1.06
		S33	0.12	0.60
		S34	0.23	0.60
	JC	S57	1.41	1.92

20 <sup>1</sup> Neutralization index =  $\log_{10}$  reduction in virus titer following neutralization with the cognate antibody, determined by radioimmuno-  
focus reduction assay (S.M. Lemon et al, J. Infect. Dis. 148:1033-1039 (1983))

25 <sup>2</sup> Neutralization index of parent HML75 virus in same assay.

To determine whether the substitution at asp-70 of VP3 conferred resistance to other monoclonal antibodies (see Example 1), S30 was tested for neutralization resistance to nine other monoclonal antibodies, including B5-B3 (see Example 3). Although the degree of neutralization varied with different antibodies, S30 was relatively resistant to each. Neutralization resistance was most evident with monoclonal antibodies having activities similar to the cognate K2-4F2 in competition studies (813, 6A5, 10.09 and 1B9; see Example 1), but S30 was at least partially resistant to each of the antibodies tested.

Asp-70 of VP3 is, therefore, involved in the antigenic site recognized by each of these monoclonal antibodies, and is thus part of an immunodominant antigenic site of HAV. While the mutation at asp-70 of VP3 of HAV might hypothetically have resulted in capsid conformational changes preventing neutralization by antibodies binding at distant sites, such an interpretation conflicts with crystallographic evidence that mutations conferring neutralization resistance in poliovirus and rhinovirus cluster at specific surface sites most likely representing the relevant epitopes (M.D. Rossman et al, Nature 317:145-153 (1985); J.M. Hogle et al, Science 229:1358-1365 (1985)).

Given in FIGURES 1A and 1B is the nucleotide sequence of VP1 of HM175 strain HAV (circled residue is ser-102 of predicted amino acid sequence). Figures 2A and 2B show the nucleotide sequence of VP3 of HM175 strain HAV (circled residue is asp-70 of predicted amino acid sequence).

SUBSTITUTE SHEET

Synthetic peptides of the instant invention derived from the asp-70 region of VP3 comprise, for example, an amino acid sequence that includes IPTLAAQFPFNASDSVGQ, DSVGQQ, SDSVGQ, ASDSVG, NASDSV, FNASDS, PFNASD or a portion of any one of these sequences that is capable of inducing neutralizing antibodies.

Peptides of the invention can be synthesized either chemically (R.B. Merrifield, J.A.C.S., Vol. 83, 1963, pp. 2149-2154) or using recombinant techniques (B.E. Clarke et al, Nature, Vol. 330, 1987, pp. 381-384).

Carrier molecules to which the synthetic peptides of the instant invention can be covalently linked (conjugated) are, advantageously, non-toxic, pharmaceutically acceptable and of a size sufficient to produce an immune response in mammals. An example of a suitable carrier molecule is the hepatitis B core protein (B.E. Clarke et al, Nature, 330:381-384 (1987)). Other suitable carrier molecules well known in the art can also be used.

The synthetic peptides can also be administered with a pharmaceutically acceptable adjuvant, for example, alum.

Linkage of a carrier molecule to a synthetic peptide of the invention can be direct or through a spacer molecule. Spacer molecules are, advantageously, non-toxic and reactive. Suitable spacer molecules include those well known in the art.

Linkage of the carrier molecule to the synthetic peptide can be accomplished using a coupling agent. Advantageously, the heterofunctional coupling agent M-maleimidobenzoyl-N-hydroxysuccinimide ester or the water soluble

compound m-maleimido-benzoylsulfosuccinimide ester is used (Green et al, Cell 28:477 (1982)).

The present invention also relates to a vaccine against HAV infection comprising, in addition to the above-described synthetic peptides having an amino acid sequence corresponding to the region of VP3 containing the asp-70 residue (examples of such sequences being given above), an additional immunogenic sequence(s) from the HAV capsid which, advantageously, is a synthetic peptide having an amino acid sequence corresponding to the region of VP1 containing the ser-102 residue. Such synthetic peptides comprise, for example, a sequence of amino acids that includes TPTFNS, FTFNSN, TFNSNN, FNSNNK, NSNNKE, SNNKEY, or a portion of any one of these sequences.

The present invention also relates to a method of producing immunity to HAV in a mammal comprising administering to a mammal at least one of the above-described conjugates in an amount sufficient to induce the production of neutralizing antibodies against HAV.

In addition to being used as a vaccine or a component of a vaccine, the synthetic peptides of the instant invention can also be used for diagnostic purposes. Peptides of the instant invention can be used, for example, in standard enzyme linked immunosorbent assays or radioimmunoassays to detect the presence of HAV antibodies.

The following nonlimiting examples illustrate the invention in more detail.

Example 1COMPETITION BETWEEN MONOCLONAL ANTIBODIES FOR  
ATTACHMENT TO HAV FIXED TO A SOLID-PHASE SUPPORT

Gradient-purified HM175 strain HAV was  
5 bound to polyclonal human convalescent antibody  
coating wells of a polyvinylchloride microtiter  
plate (J.T. Stapleton et al, J. Virol. 61:491-498  
(1987)) Dilutions of ten different murine  
monoclonal antibodies were added to the virus-coated  
10 wells prior to the addition of much smaller  
quantities of [ $^{125}$ I]-labelled monoclonal antibody  
K3-4C8 or B5-B3 (see ~~solid~~<sup>diagonally stripped</sup> and stippled columns,  
respectively, in Figure 3). The quantity of  
radiolabelled antibody bound to virus was compared  
15 with the quantity bound in the absence of any  
competing antibody (see Figure 3). Results obtained  
with radiolabelled K3-4C8 were similar to those  
obtained with radiolabelled K2-4F2. The source of  
the monoclonal antibodies was as follows: K3-4C8,  
20 K3-2F2 and K2-4F2, Commonwealth Serum Institute,  
Melbourne, Australia (A. MacGregor et al, J. Clin.  
Microbiol. 18:1237-1243 (1983)); 6A5, 1B9, 2D2 and  
3E1, J. Hughes and E. Emini, Merck Institute of  
Therapeutic Research, West Point, PA, USA (J.V.  
25 Hughes et al, J. Virol. 52:465-473 (1984)); 813 and  
10.09, D. Crevat and E. Deloince, Clonatec, Paris,  
France; and B5-B3, R. Tedder, Middlesex Hospital,  
London, U.K.

SUBSTITUTE SHEET



Example 2

## MAP OF GENOMIC REGIONS

The map of genomic regions from various HAV neutralization-escape mutants was sequenced by the dideoxynucleotide method (D.M.A. Evans et al, Nature 304:459-462 (1983); F. Sanger et al, Proc. Nat. Acad. Sci. USA 74:5463-5467 (1977)). The genomic region encoding the capsid proteins of HM175 strain HAV is depicted at the top of Figure 4, with nucleotide positions and putative peptide cleavage sites displayed according to Cohen et al (J.I. Cohen et al, J. Virol. 61:50-59 (1987)). At the right of Figure 4, the various HM175 variants included in this study (S1 through S57) are listed along with the parent virus HM175, while to the left are shown the antibodies against which these variants were selected. JC was polyclonal human convalescent antibody, while the other antibodies were murine monoclonal ascitic fluids. Solid lines indicate RNA regions sequenced, while vertical arrows indicate the location of identified nucleotide base substitutions. Neutralization-escape variants of HM175 virus were isolated as described previously (J.T. Stapleton et al, J. Virol. 61:491-498 (1987)), and amplified in 850 cm<sup>2</sup> roller bottle cultures of BS-C-1 cells in the absence of antibody (to conserve antibody). Virus was assessed for continued neutralization-resistance or reversion to neutralization susceptibility at two weeks by radioimmunofocus reduction assay (see Table 1; J.T. Stapleton et al, J. Virol. 61:491-498 (1987); S.M. Lemon et al, J. Infect. Dis. 148:1033-1039 (1983)) and harvested by freeze-thawing of cells at three

weeks. After purification of virus on hybrid sucrose-cesium chloride gradients (J. de Chastonay et al, Virology 157:268-275 (1987)), virus was treated with proteinase K/sodium dodecyl sulfate (J.R. Ticehurst et al, Proc. Nat. Acad. Sci. USA 80:5885-5889 (1983)) and viral RNA was extracted with phenol-chloroform. 2-10 ng purified viral RNA was annealed with 13.5 ng HAV-specific oligonucleotide primers and sequenced by reverse transcriptase-mediated primer-extension (D.M.A. Evans et al, Nature 304:459-462 (1983); F. Sanger et al, Proc. Nat. Acad. Sci. USA 74:5463-5467 (1977)) in the presence of dideoxynucleotides and high specific activity [<sup>35</sup>S]-labelled deoxynucleotides (1000-1500 Ci/mole each). Reaction products were separated on 8% acrylamide, 7.6 M urea, Tris-borate-EDTA gradient gels. The sequence was compared with that derived from cDNA clones of cell culture-adapted HMI75 virus.

20

Example 3

RESISTANCE OF MUTANT S30 TO NEUTRALIZATION  
BY NINE DIFFERENT MONOCLONAL ANTIBODIES TO HAV

The HAV variant S30 was selected by repeated neutralization and passage in the presence of antibody K2-4F2 (J.T. Stapleton et al, J. Virol. 61:491-498 (1987)) and has a mutation at base position 1677 leading to substitution of asp-70 of VP3 with his. Neutralization resistance was assessed by the radioimmunofocus reduction method (S.M. Lemon et al, J. Infect. Dis. 148:1033-1039 (1983)) using a chloroform-extracted, 0.1% sodium lauryl sarcosinate-treated virus inoculum of

approximately 4000 radioimmunofocus-forming units/ml and a  $10^{-1}$  or  $10^{-2}$  dilution of antibody. Results are presented as  $\log_{10}$  reductions in virus titer and are shown for S30 virus as stippled columns, and for plaque-purified parent HML75 virus as ~~solid~~ <sup>diagonally striped</sup> columns (see Figure 5). Previous studies demonstrated that S30 virus was also resistant to neutralization by K3-2F2 (J.T. Stapleton et al, J. Virol. 61:491-498 (1987)).

The foregoing invention has been described in some detail by way of examples for purposes of clarity and understanding. It will be obvious to those skilled in the art from a reading of the disclosure that the synthetic peptides of the instant invention may differ slightly in amino acid sequence without departing from the scope of the invention. It will also be obvious that various combinations in form and detail can be made without departing from the scope of the invention.

WHAT IS CLAIMED IS:

1. A synthetic peptide capable of producing an immunogenic response to the viral causative agent of hepatitis A comprising a chain of amino acids having a sequence homologous to a portion of the amino acid sequence of a neutralization immunogenic site of the VP3 structural protein of the HAV capsid, which peptide is capable of inducing in a mammal neutralizing antibodies against HAV.
2. The peptide according to claim 1, wherein said peptide comprises an amino acid sequence corresponding to a region of VP3 containing asp-70.
3. The peptide according to claim 2, which peptide comprises the sequence DSVGQQ.
4. The peptide according to claim 2, which peptide comprises the sequence SDSVGQ.
5. The peptide according to claim 2, which peptide comprises the sequence ASDVGV.
6. The peptide according to claim 2, which peptide comprises the sequence NASDSV.
7. The peptide according to claim 2, which peptide comprises the sequence FNASDS.
8. The peptide according to claim 2, which peptide comprises the sequence PFNASD.

9. The peptide according to claim 2, which peptide comprises the sequence IPTLAAQFPFNASDSVGQ.

10. An immunogenic conjugate capable of inducing in a mammal the production of high titers of neutralizing antibodies against HAV, said  
5 conjugate comprising:

(i) a carrier molecule covalently linked to (ii) a synthetic peptide consisting essentially of a chain of amino acids having a sequence  
10 homologous to a portion of the amino acid sequence of a neutralization immunogenic site of the VP3 structural protein of the HAV capsid containing asp-70.

11. The conjugate according to claim 10,  
15 further comprising a synthetic peptide having an amino acid sequence homologous to a portion of the amino acid sequence of an immunogenic site of the VP1 structural protein of the HAV capsid containing ser-102.

12. The conjugate according to claim 10,  
20 wherein said carrier molecule is hepatitis B core protein.

13. The conjugate according to claim 10,  
wherein said carrier molecule is covalently linked  
25 to said peptide through at least one spacer molecule.

14. A method of producing immunity to HAV in a mammal comprising administering to said mammal at least one conjugate according to claim 10 in an

amount sufficient to induce the production of neutralizing antibodies against HAV.

15. A method of determining the presence and titers in mammalian serum of neutralizing antibodies against HAV comprising the steps of:

(i) contacting a synthetic peptide consisting essentially of a chain of amino acids having a sequence homologous to a portion of the amino acid sequence of a neutralization immunogenic site of the VP3 structural protein of the HAV capsid containing asp-70, with antibodies from mammalian serum; and

(ii) measuring the formation of complexes between said peptide and said antibody.

16. A DNA segment encoding a sequence of amino acids homologous to a portion of the amino acid sequence of a neutralization immunogenic site of the VP3 structural protein of the HAV capsid, which peptide is capable of inducing in a mammal neutralizing antibodies against HAV.

1/7

## FIG. 1A

LIMITS: 2207 3106

Nucleotide sequence VP1 of HM175 strain HAV; underlined residue is ser-102 in predicted amino acid sequence.

GTT V	GGA G	GAT D	GAT D	TCT S	GGA G	GGT G	TTT F	TCA S	ACA T	ACA T	TCT S	GTT V	GAA E	CAG Q	AAT N	GTT V	CCA P	2266 GAT D
CCC P	CAA Q	GTT V	GGT G	ATA I	ACA T	ACC T	ATG M	AAA K	GAT D	TTG L	AAA K	GCT A	AAA K	AAC N	AGA R	GGG G	AAA K	2326 ATG M
GAT D	GTT V	TCA S	GGA G	GTA V	CAA Q	GCA A	CCT P	GTG V	GGG G	GCT A	ATC I	ATT I	ACA T	GAG E	GAT D	CCA P	GTT V	2386 TTA L
GCA A	AAG K	AAA K	GTA V	CCT P	GAG E	ACA T	TTT F	CCT P	GAA E	TTG L	AAA K	TTG L	GGA G	TCC S	AGA R	CAT H	ACA T	2446 TCA S
GAT D	CAT H	ATG M	TCC S	ATC I	TAC Y	AAG K	TTT F	ATG M	GGG G	AGG R	TCT S	CAT H	TTT F	TGC C	ACT T	TTT F	ACA T	2506 TTC F
AAT N	TCA S	AAT N	AAT N	AAA K	GAG E	TAC Y	ACA T	TTT F	CCT P	ATA I	ACC T	TTG L	TCT S	ACC T	TCT S	AAT N	CCT P	2566 CCT P
CAT H	GGT G	TTG L	CCA P	TCA S	ACA T	CTG L	AGG R	TGG W	TTT F	TTC F	AAC N	TTG L	TTT F	TTG L	TAT Y	AGA R	GGG G	2626 CCT P

TO FIG. 1B

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FROM FIG. 1A

TTA	GAT	CTG	ACA	ATT	ATT	ACA	GGA	GCA	ACT	GAT	GTA	GAT	GGC	ATG	GCC	TGG	2686
L	D	L	T	I	I	T	G	A	T	D	V	D	G	M	A	W	ACT
																F	T
CCA	GTA	GGT	CTT	GCC	GTT	GAT	ACT	TGG	GTA	GAG	AAG	GAG	TCA	GCT	TTG	TCT	2746
P	V	G	L	A	V	D	T	W	V	E	K	E	S	A	L	S	GAC
																I	D
TAC	AAA	ACT	GCT	CTT	GGA	GCT	GTC	TTT	AAC	ACA	AGG	AGA	ACA	GGG	AAC	ATT	2806
Y	K	T	A	L	G	A	V	F	N	T	R	R	T	G	N	I	ATT
																Q	I
AGA	TTA	CCA	TGG	TAT	TCT	TAT	TTA	GCT	GTG	TCT	GGG	GCA	CTG	GAT	GGT	TTG	2866
R	L	P	W	Y	S	Y	L	Y	V	S	G	A	L	D	G	L	GAC
																G	D
AAG	ACA	GAT	TCT	ACA	TTT	GGA	TTG	TCT	ATT	CAG	ATT	GCA	AAT	TAC	AAT	CAT	2926
K	T	D	S	T	F	G	L	V	I	Q	I	A	N	Y	N	H	GAT
																S	D
AGAA	TAC	TTG	TCT	TTT	AGT	TGT	TAT	TCT	GTC	ACA	GAA	CAA	TCA	GAG	TTT	TAT	2986
E	Y	L	S	F	S	C	Y	L	V	T	E	Q	S	E	F	Y	CCC
																P	
AGA	GCT	CCA	TTG	AAC	TCA	AAT	GCC	TTA	TCC	ACT	GAA	TCA	ATG	ATG	AGC	AGA	3046
R	A	P	L	N	S	N	A	L	S	T	E	S	M	M	S	R	GCA
																I	A
GCT	GGA	GAC	TTG	GAG	TCA	TCA	GTG	GAT	CCT	AGA	TCA	GAG	GAA	GAT	AAA	AGA	3106
A	G	D	L	E	S	S	V	D	P	R	S	E	E	D	K	R	GAG
																F	E

FIG. 1B



## FIG. 2A

LIMITS: 1469 2206

Nucleotide sequence of VP3 of HM175 strain HAV; underlined residue is asp-70 in predicted amino acid sequence.

ATG	ATG	AGA	AAT	GAA	TTT	AGG	GTC	AGT	ACT	ACT	GAG	AAT	GTG	GTG	AAT	CTG	TCA	AAT	1528
M	M	R	N	E	F	R	V	S	T	T	E	N	V	V	N	L	S	N	TAT
																		Y	
GAA	GAT	GCA	AGA	GCA	AAG	ATG	TCT	TTT	GCT	TTG	GAT	CAG	GAA	GAT	TGG	AAA	TCT	GAT	1588
E	D	A	R	A	K	M	S	F	A	L	D	Q	E	D	W	K	S	D	CCG
																		P	
TCC	CAG	GGT	GGT	GGG	ATC	AAA	ATT	ACT	CAT	TTT	ACT	ACT	TGG	ACA	TCT	ATT	CCA	ACT	1648
S	Q	G	G	G	I	K	I	T	H	F	T	T	W	T	S	I	P	T	TTG
																		L	
GCT	GCT	CAG	TCT	TTT	CCA	TTT	GCT	TCA	GAC	TCA	GTT	CGT	CAA	CAA	ATT	AAA	GTT	ATT	1708
A	A	Q	F	F	P	N	A	S	<u>D</u>	S	V	G	Q	Q	I	K	V	I	CCA
																		P	
GTT	GAC	CCA	TAT	TTT	TTT	CAA	ATG	ACA	AAT	ACA	AAT	CCT	GAC	CAA	AAA	TGT	ATA	ACT	1768
V	D	P	Y	F	F	Q	M	T	N	T	N	P	D	Q	K	C	I	T	GCT
																		A	
TTG	GCT	TCT	ATT	TGT	CAG	ATG	TTT	TGT	TTT	TGG	AGA	GGA	GAT	CTT	GTC	TTT	GAT	TTT	1828
L	A	S	I	C	Q	M	F	C	F	W	R	G	D	L	V	F	D	F	CAA
																		Q	
GTT	TTT	CCC	ACC	AAA	TAT	CAT	TCA	GGT	AGA	TTA	CTG	TTT	TGT	TTT	GTT	CCT	GGC	AAT	1888
V	F	P	T	K	Y	H	S	G	R	L	L	F	C	F	V	P	G	N	GAG
																		E	

TO FIG. 2B

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FROM FIG. 2A

CTA L	ATA I	GAT D	GTT V	TCT S	GGA G	ATC I	ACA T	TTA L	AAG K	CAA Q	GCA A	ACT T	ACT T	GCT A	CCT P	TGT C	GCA A	GTA V	ATG M	1948
GAT D	ATT I	ACA T	GGA G	GTG V	CAG Q	TCA S	ACT T	TTG L	AGA R	TTT F	CGT R	GTT V	CCC P	TGG W	ATT I	TCT S	GAC D	ACT T	CCT P	2008
TAC Y	AGA R	GTG V	AAC H	AGG R	TAT Y	ACA T	AAG K	TCA S	GCA A	CAT H	CAG Q	AAA K	GGT G	GAG E	TAC Y	ACT T	GCC A	ATT I	GGG G	2068
AAG K	CTT L	ATT I	GTG V	TAT Y	TGT C	TAT Y	AAC N	AGA R	TTG L	ACC T	TCT S	CCT P	TCT S	AAC N	GTT V	GCT A	TCC S	CAT H	GTC V	2128
AGA R	GTG V	AAT N	GTT V	TAT Y	CTT L	TCA S	GCA A	ATT I	AAC N	TTG L	GAA E	TGT C	TTT F	GCT A	CCT P	CTT L	TAT Y	CAT H	GCT A	2188
ATG M	GAT D	GTT V	ACT T	ACA T	CAA Q															

FIG. 2B

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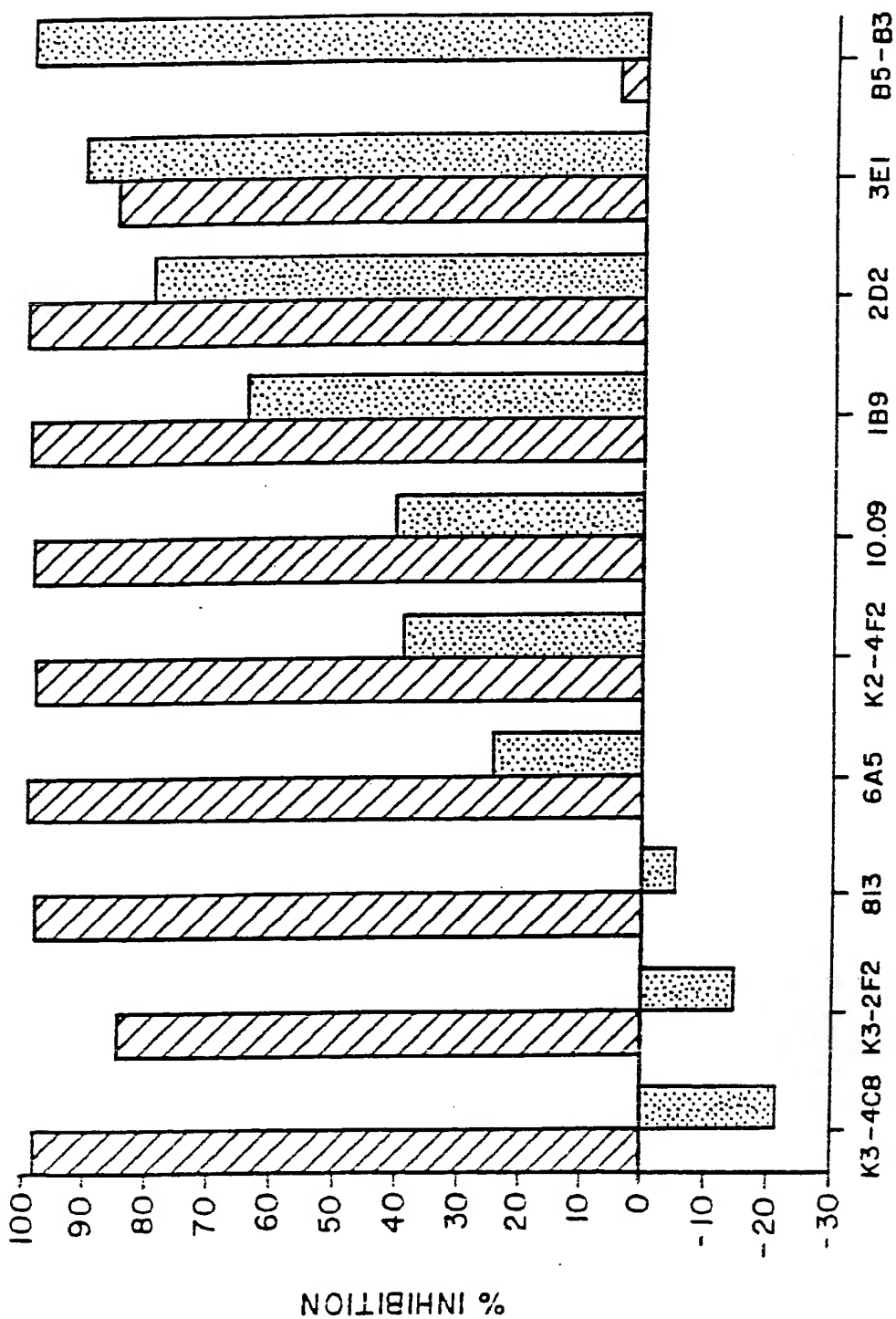


FIG. 3

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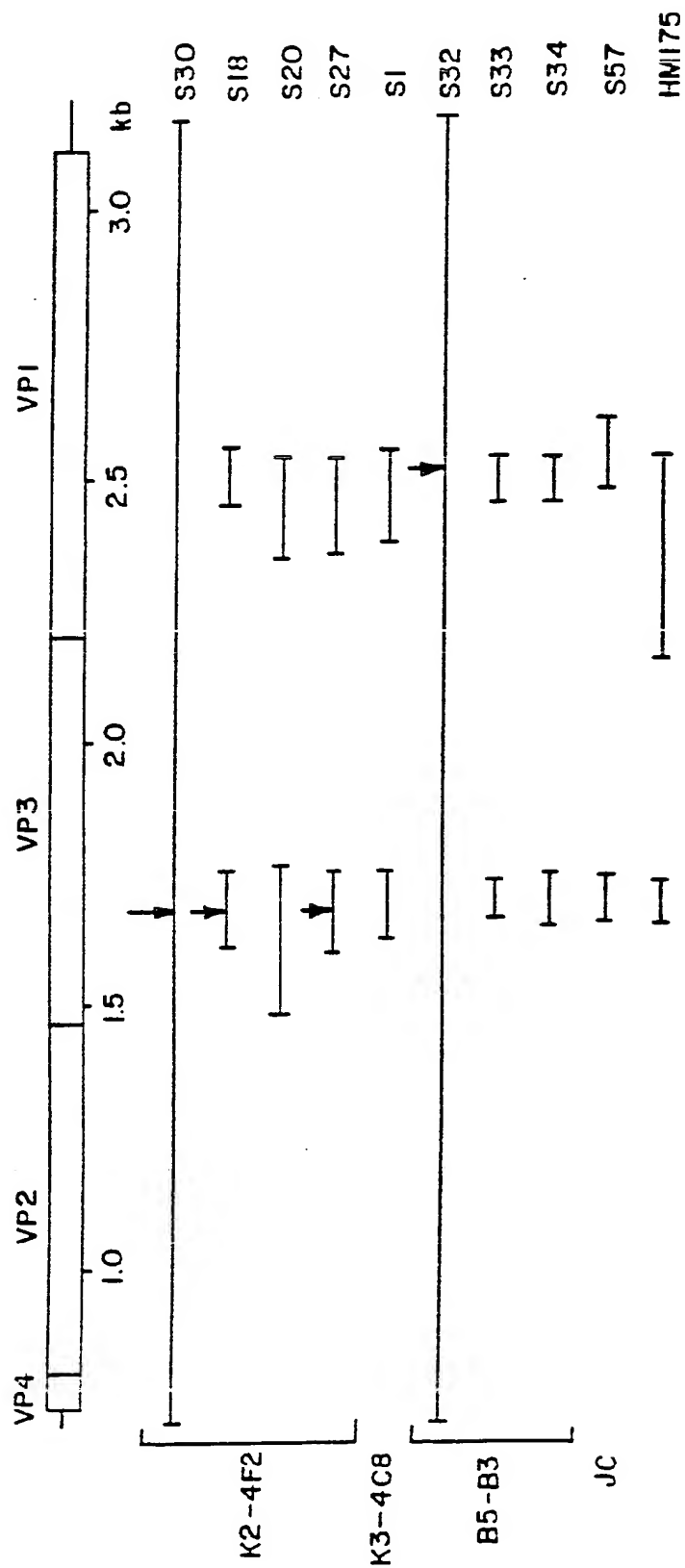


FIG. 4

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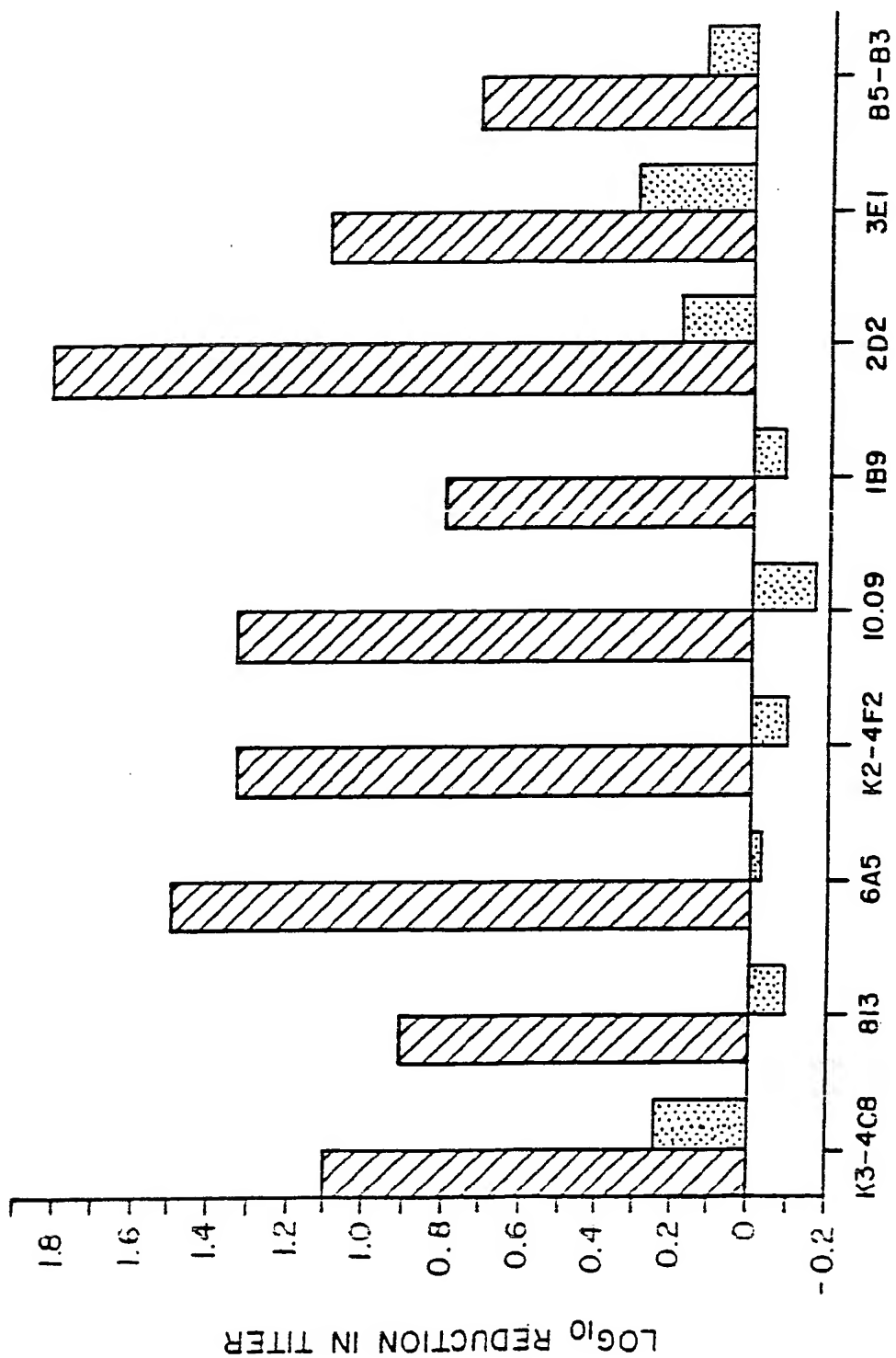


FIG. 5

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# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/00097

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
INT. CL. 4th Ed. C07K 7/06, 08, 10; 13/00		
U.S. CL. 530/324, 325, 326, 327, 328, 329, 350; 424/88; 435/6, 68		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
U.S.	530/324, 325, 326, 327, 328, 329, 350; 424/88; 435/6, 68	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b>		
Category <sup>*</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X	Journal of General Virology, Vol. 57, Issued 1981 (Gunter) "The Physicochemical Properties of Infectious Hepatitis A Virions". pages 331-341 (See summary).	1-6
X	Journal of Virology, Vol. 52, No. 2, Issued 1984 (Hughes) "Neutralizing Monoclonal Antibodies to Hepatitis A Virus: Partial Localization of a Neutralizing Antigenic Site". pages 465-473 (See summary).	1-16
X	Journal of Virology, Vol. 55, No. 2, Issued 1985 (Hughes) "Isolation and Immunizations with Hepatitis A Viral Structural Proteins: Induction of Antiprotein, Antiviral, and Neutralizing Responses". pages 395-401 (See summary).	1-16
<p><sup>*</sup> Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"Δ" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
21 April 1989	08 JUN 1989	
International Searching Authority	Signature of Authorized Officer	
ISA/US	DELBERT R. PHILLIPS	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE FIRST SHEET)		
Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Journal Virology, Vol. 58, Issued 1986 (Wheeler) "Structure of the Hepatitis A Virion: Peptide Mapping of the Capsid Region". pages 307-313 (See summary).	1-16
X	Chemical Abstract, Vol. 102, Issued 24 June 1985 (Columbus, OH.) "Molecular cloning and partial sequencing of hepatitis A Viral cDNA". abstract no. 216279v (Linemeyer)	1-16
X,P	Chemical Abstract, Vol. 109, Issued 19 December 1988 (Columbus, OH.) "Identification of an immunodominant antigenic site involving the capsid protein VP3 of hepatitis A Virus". (Ping) Abstract no. 228155j.	1-16
X	Biological Abstract, Vol. 83, Issued 01 March 1987 "The genomic map of hepatitis A Virus: An Alternate analysis". (Diamond) Abstract no. 50184.	1-16
X	Biological Abstract, Vol. 77, Issued 01 February 1984 "Topology and immunoreactivity of capsid proteins in hepatitis A Virus". (Gerlich) Abstract no. 24102	1-16
X	US,A, 4,744,983 (Morein) Published 17 May 1988 (See Abstract).	1-16
X	US,A, 4,614,793 (Hughes) Published 30 September 1986 (See Abstract).	1-16
X	US,A, 4,578,269 (Morein) Published 25 March 1986 (See Abstract).	1-16